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(54) **GENE DU CHROMOSOME 1P ET PRODUITS GENETIQUES
ASSOCIES A LA MALADIE D'ALZHEIMER**

(54) **CHROMOSOME 1P GENE AND GENE PRODUCTS RELATED
TO ALZHEIMER'S DISEASE**

(57) La présente invention divulgue des molécules d'acide nucléique codant des gènes qui interviennent dans les troubles du SNC, notamment dans la maladie d'Alzheimer et dans l'épilepsie. L'invention divulgue également des produits génétiques, des vecteurs et des cellules hôtes appropriés pour l'expression de produits génétiques de ce type. Des méthodes sont présentées pour déceler la présence d'un gène impliqué dans la maladie d'Alzheimer chez l'homme et pour le traitement des personnes souffrant de la maladie d'Alzheimer. De plus, on divulgue des marqueurs qui sont associés à la maladie d'Alzheimer. Enfin, on présente également des méthodes sous forme de trousse pour la détection de la maladie d'Alzheimer chez un sujet.

(57) The present invention discloses nucleic acid molecules encoding genes involved in CNS disorders such as Alzheimer's disease and epilepsy. Also disclosed are gene products, vectors and host cells suitable for expression of such gene products. Methods are provided for detecting the presence of a gene involved in Alzheimer's disease in human subjects and for treating humans suffering from Alzheimer's disease. Furthermore, markers which are associated with Alzheimer's disease are disclosed. Methods in kits for the detection of Alzheimer disease in a subject are additionally provided.



ABSTRACT OF THE DISCLOSURE

The present invention discloses nucleic acid molecules
5 encoding genes involved in CNS disorders such as Alzheimer's disease
and epilepsy. Also disclosed are gene products, vectors and host cells
suitable for expression of such gene products. Methods are provided for
detecting the presence of a gene involved in Alzheimer's disease in
human subjects and for treating humans suffering from Alzheimer's
10 disease. Furthermore, markers which are associated with Alzheimer's
disease are disclosed. Methods in kits for the detection of Alzheimer
disease in a subject are additionally provided.

TITLE OF THE INVENTION

CHROMOSOME 1p GENE AND GENE PRODUCTS
RELATED TO ALZHEIMER'S DISEASE

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FIELD OF THE INVENTION

This invention relates generally to central nervous system (CNS) disorders. More particularly, this invention relates to Alzheimer's disease. In addition the invention relates to the diagnosis
10 and treatment of Alzheimer's disease.

BACKGROUND OF THE INVENTION

Alzheimer's disease (AD) is the most common cause of progressive cognitive decline in the aged population. It causes 100 000
15 deaths each year in the United States where it is the fourth leading cause of death. Alzheimer described amyloid plaques, neurofibrillary tangles and dementia that characterize AD in 1907. The usual presenting symptoms are deficits of recent memory often in association with language and visuospatial and attention problems.

20 To date, three genes have been identified that, when mutated, can lead to early onset forms of AD and variation in a fourth one has been implicated as a risk or susceptibility factor for AD.

β-amyloid precursor protein

The major protein of the senile plaques is β-amyloid

cortex. APP was the first gene in which mutations were found to cause familial Alzheimer's disease (FAD). The APP gene, located on chromosome 21, has 19 exons and A β is encoded by parts of exons 16 and 17 (Lemaire et al., 1989). Four mutations in the APP gene have 5 been described (Chartier-Harlin et al., 1991; Fidani et al., 1992; Goate et al., 1991; Karlinsky et al., 1992; Mullan et al., 1992; Murrell et al., 1991; Naruse et al., 1991; but they account for only 5% of published early-onset FAD).

Presenilins

10 In 1992, Schellenberg et al (Schellenberg et al., 1992) reported a second locus causing early-onset AD on chromosome 14q24.3. A positional cloning strategy permitted the identification of a candidate gene, the S182 gene (Sherrington et al., 1995) later renamed presenilin-1 or *PS1*, that carried coding region mutations in families 15 multiply affected by early-onset AD. The *PS1* gene, composed of 10 exons, encodes a 467 amino acids protein with 7 to 10 transmembrane domains. More than 35 different mutations have been found in the *PS1* gene in over 50 families of different ethnic origins (see van Broeckhoven, 1995 for review). The proportion of early-onset familial AD cases due to 20 mutations in the *PS1* gene is around 50%.

A genome-wide search conducted on another population with familial early-onset AD indicated another locus on chromosome 1 (Levy-Lahad et al., 1995a). The chromosome 1 FAD gene was cloned by virtue of its homology to *PS1*. The *PS2* gene is composed of 12 exons 25 and encodes a 448 amino acids protein (Levy-Lahad et al., 1995b). It

rare cause of FAD protein (Levy-Lahad *et al.*, 1995b; Rogaev *et al.*, 1995).

APO e4

The apolipoprotein E (APOE) gene, located on 5 chromosome 19q13.2 has been identified as a susceptibility factor for AD by genetic analysis of late-onset FAD pedigrees (Pericak-Vance *et al.*, 1991). APO E is a major serum lipoprotein involved in cholesterol metabolism. Three common isoforms of APOE are encoded by alleles e2, e3, and e4 as a result of amino acids changes at codons 112 and 10 158. The APO e4 allele shows a dose dependent increase in risk for AD, apparently mediated through a decrease in the age of onset of disease (Corder *et al.*, 1993).

Not everyone having the susceptibility e4 allele will 15 develop illness and many who lack the allele will also develop AD. APOE testing is therefore not useful for predicting whether someone will develop AD.

Research on the molecular ethiology of the a complex 20 disease such as Alzheimer disease has been confounded by the large number of hereditary and environmental factors involved and by the paucity of neuropathological and neurochemical studies on brains for affected individual. The finding of a linked marker involved in one hereditary form of Alzheimer disease will help to resolve the number of different genes underlying this complex disease. This markers can be used eventually to provide genetic counselling in some affected families. 25 Most importantly, the delineation of the genomic region containing Alzheimer disease gene will provide a mean to eventually discover and characterize this gene(s) in its encoded protein(s). The finding of link-

markers will also make it possible to evaluate the role of gene(s) in this chromosomal region in the different levels of severity and onset of Alzheimer's disease.

5 **SUMMARY OF THE INVENTION**

The invention seeks to provide diagnosis and therapeutic tools for CNS disorders. Particularly the invention seeks to provide diagnosis and therapeutic tools for Alzheimer's disease (AD). Herein, the term AD-related nucleic acid is not meant to be restricted to AD only, 10 since other CNS disorders are herein shown to share common genes and products thereof.

The present invention seeks to provide a nucleic acid segment isolated from human comprising at least a portion of a gene responsible for CNS disorders and particularly to AD. The AD-related nucleic acid segment can be isolated using conventional methods which 15 include for example YAC and BAC cloning, exon trapping and the like. Such nucleic acids could also be synthesized chemically. Having the AD-related nucleic acid segments of the present invention, parts thereof or oligos derived therefrom, other AD-related sequences using methods 20 described herein or other well known methods.

The invention also seeks to provide prokaryotic and eukaryotic expression vectors harboring the AD-related nucleic acid segment of the invention in an expressible form, and cells transformed with same. Such cells can serve a variety of purposes such as *in vitro* 25 models for the function of AD-related genes as well as for screening pharmaceutical compounds that could regulate the expression of the gene or the activity of the protein encoded therefrom. For example, such

a cell, expressing a DNA sequence encoding a protein involved in proper neural function through the inositol phosphate pathway could serve to screen for pharmaceutical compounds that regulate neural function or inositol phosphate pathway.

5 An expression vector harboring AD-related nucleic acid segment or part thereof, can be used to obtain substantially pure protein. Well-known vectors can be used to obtain large amounts of the protein which can then be purified by standard biochemical methods based on charge, molecular weight, solubility or affinity of the protein or 10 alternatively, the protein can be purified by using gene fusion techniques such as GST fusion, which permits the purification of the protein of interest on a glutathione column. Other types of purification methods or fusion proteins could also be used.

15 Antibodies both polyclonal and monoclonal can be prepared from the protein encoded by the Ad-related nucleic acid segment of the invention. Such antibodies can be used for a variety of purposes including affinity purification of the AD-related protein and diagnosis of a predisposition to AD or other CNS disorders.

20 The AD-related nucleic acid segment, parts thereof or oligonucleotides derived therefrom, can further be used to identify differences between AD affected individuals and non AD-affected individuals. Similarly such segments can be used to identify a predisposition to AD in individuals. The AD-related sequences can further be used to obtain animal models for the study of CNS disorders.

25 Transgenic animals can be obtained. The functional activity of the AD

The human AD-related sequences can be used in a DNA-based diagnostic assay to identify these individuals in the population who are at risk for the above mentioned types of diseases.

Further, the present invention seeks to provide the use of 5 the AD-related protein as a pharmacological target for modulating neuronal function and the like.

As used herein in the specifications and appended claims, the term "oligonucleotide" includes both oligomers of ribonucleotides and oligomers of deoxyribonucleotides.

10 The term high stringency hybridization conditions, as used herein and well known in the art, includes, for example: 5 X SSPE (1 X SSPE is 10 mM Na-phosphate, pH 7.0; 0.18 M NaCl; 1 mM Na₂ EDTA), 5 x Denhardt's solution (from a 100 X solution containing 2% BSA, 2% Ficoll, 2% polyvinyl pyrrolidone), 0.1% SDS, and 0.5 mg/ml denatured 15 salmon sperm DNA, at 65°C. Other conditions considered stringent include the use of formamide. An example of washing conditions for the blot includes, as a final stringency wash, an incubation of the blot at 65°C in 0.1 X SSPE, 0.1% SDS for 1 hour.

20 In the specifications and appended claims, it is to be understood that absolute complementarity between the primers and the templat is not required. Any ligonucleotide having a sufficient complementarity with the t mplat , so that a stabl duplex is formed, is suitabl . Since th formation of a stable duplex depends on th sequence and length of the oligonucleotide and its complementarity t 25 the templat it hybridizes to, as well as th hybridization conditions, on skilled in the art may readily determine the degree of mismatching that

can be tolerated between the oligonucleotide and its target sequence for any given hybridization condition.

The invention features the means to identify factors that modulate the transcriptional activity of AD-related genes. Such factors 5 include, without being limited thereto, other kinases, phosphatases, nuclear receptors and transcriptionally regulatory proteins.

The present invention is also related to the use of AD-related sequences of the present invention and functional derivatives thereof to screen for agents that modulate gene expression or the activity 10 of the products of these segments. Such modulators can be used as lead compounds to design or search drugs that can modulate the level of expression of these genes or the activity of their products.

Further, the present invention concerns a method for measuring the ability of a compound to act as an agonist or antagonist of 15 AD-related gene products comprising (a) contacting the compound with a transfected host cell expressing an AD-related sequence or mutant thereof, and (b) comparing the level of activity of the product thereof or the level of expression of the AD related sequence. It is herein contemplated to use the control regions of AD-related nucleic acids 20 hooked to heterologous genes such as any appropriate reporter gene (i.e. luciferase, chloramphenicol acetyl transferase, green fluorescent protein or β -galactosidase).

The invention is based on the results of an association study in recently founded populations in which a linkage disequilibrium mapping of Alzheimer's disease was carried out. This analysis permitted 25 the construction of haplotypes and enabled the identification of additional

markers in the vicinity of the most significant markers identified by the association analysis.

From these data, it was inferred that the Alzheimer's disease loci comprise D10S212, D6S273, D1S228, D1S232, Gata89a1, 5 D2S126, and D8S552.

Now that the location of Alzheimer's disease markers have been identified, other markers can be found using methods known in the art. Generally, primers are utilized which will identify markers associated with Alzheimer disease, for example (GD)n and RFLP markers.

10 The invention also extends to products useful for carrying out the assay, such as DNA probes (labelled or unlabelled), kits, and the like.

As broadest, the invention comprises detecting: the presence of genes involved in Alzheimer's disease by analysing human 15 chromosomes, particularly chromosome 10, 6, 1, 9, 2 and 8 for further markers or DNA polymorphisms or the like linked to Alzheimer's disease.

The use RFLP's is only one preferred embodiment of detecting the polymorphisms. The most common methodology for detecting the presence of RLFP is to carry out restriction analysis using 20 a given enzyme, perform a Southern procedure with a desired probe and identify a given RFLP or RFLPs. The use RLFPs in linkag analysis and genetic testing is well known in th art (for exampl , see Gusella, US 4, 666,828 incorporated herein by reference in Donnus-Kell r t al., 1987, C II. 51:319-337). It should be clear that other methods t identify 25 differences at the DNA l vel, or RNA level which are not relat d to RFLPs can also be used. These methods are well known in the art of human

be used. Techniques such as amplification of the desired regional chromosome coupled with direct sequencing, a location of polymorphisms and the chromosome by radio-labelling, fluorescent-labelling and enzyme-labelling can also be utilized.

5 DNA and/or RNA can be amplified using an amplifiable RNA sequence as a probe and q β -replicas.

The polynucleotide probes may be RNA or DNA and preferably DNA, and can be labelled by standard labelling techniques such as with the radio-label, enzyme-label, fluorescent-label, biotin-avidin 10 label and the like, which allow for the detection after hybridization as commonly known in the art.

15 Comparison of the RLFP or RLFPs for affected and unaffected individuals in the family line of the subject, with the RLFP or RLFPs (or other methods) for the subject under investigation will quickly reveal the presence or absence of the Alzheimer disease gene(s) in the subject. Results of this expresses in terms of probability of presence of the Alzheimer disease gene(s) in the subject.

20 A number of methods are available to the person of ordinary skill to obtain other genetic sequences useful for probes in accordance with the present invention. Non limiting examples of such methods include random DNA sequences which can be tested for their specificity, construction of DNA libraries and isolation of clones therefrom. The results of such methods is to identify a probe which can detect a polymorphism useful for testing for Alzheimer disease. The polymorphism 25 must be found to be linked to Alzheimer disease or the other useful markers in families studies, all to be adjacent to preexisting markers.

A particular probe can have any desired sequence as long as it is capable of identifying the polymorphism in the involved DNA regional or locus, it can be a DNA or RNA fragment, maybe synthesized chemical, enzymatically or isolated from a plasmid as well known to the 5 person of ordinary skill. If a polymorphism is found in a gene product, such as a mRNA, the presence of that polymorphic mRNA may be assayed directly with the probe, especially with antisense RNA probe.

Now that chromosomal location of the Alzheimer disease genes have been identified and defined to a small region, the region can 10 be cloned and characterized by general methods known in the art.

The method lends itself readily to the formulation of kits which can utilized in diagnosis.

Having now generally described the invention, the same will be understood by reference to certain specific examples that are provided 15 here in exemplary form only and are not intended to be limiting unless otherwise specified.

DESCRIPTION OF THE PREFERRED EMBODIMENT

GENETIC ANALYSIS

20 The study of genetic diseases in families by linkage analysis has been very useful to find the genes involved in simple genetic disorders. But for complex disorders in which genetic factors may be numerous and may be only part of the cause, family studies have given only modest results. Methods based on affected sib pairs which do not 25 necessitate knowledge of the familial inheritance pattern were successful in a few cases. Finally, association studies which are designed as case-control studies to compare unrelated affected and unaffected

individuals in a population are widely used to search for genes or genetic markers that can be associated with a disease. In some cases, a positive association can be found because some patients in the sample are distant relatives and thus share a specific variation in or around the 5 disease gene which is not widely present in the general population (referred to as linkage disequilibrium). A systematic search of the genome for such associations has been proposed, but this would require a large number of DNA markers if done on a normal population. However, it was thought that this would be feasible in recently founded populations 10 because seemingly unrelated patients are in fact related close enough that they share large segments of DNA, inherited with their disease gene from common ancestors (Houwen *et al.*, 1995). This was recently confirmed by the localization of the benign recurrent intrahepatic cholestasis (BRIC) gene in only three patients from an isolated 15 community in The Netherlands as well as for the infantile-onset spinocerebellar ataxia (IOSCA) gene in the Finnish population (Houwen *et al.*, 1995; Nikali *et al.*, 1995).

One of the practical advantages of this approach is that 20 there is no need to collect families as for linkage analysis or a large number of affected and unaffected individuals as for an association study. All that is necessary is to find distantly related affected individuals in an appropriate population, that is, one which is relatively young, descended from a relatively small number of founders, and which growth has occurred primarily via reproduction and not by immigration.

The Saguenay - Lac-Saint-Jean population

- The population of the Saguenay - Lac-Saint-Jean (SLSJ) region in Quebec (Canada) is a founder population which has the characteristics previously described. It is homogeneous from a sociocultural point of view, being 95% francophone and of catholic tradition. This is also true at the genetic level: some diseases show relatively high or low incidences in SLSJ. The reasons for the genetic homogeneity can be traced back to recent waves of immigration. SLSJ was first opened to settlement around 1840. From this time until the beginning of the 20th century, the neighboring region of Charlevoix - which itself was relatively homogeneous - provided for the majority of immigrants who settled in SLSJ. Moreover, the "familial nature" of this immigration contributed to a more favorable implantation of the people originating from Charlevoix as compared to other isolated immigrants coming from other parts of Quebec. The rapid increase of this population by natural reproduction all through the 19th century and the early part of the 20th century also contributed to its establishment as the main core of the population of the SLSJ region (Bouchard and De Braekeleer, 1991; Heyer and Tremblay, 1995).
- We have confirmed that the SLSJ population is a suitable population for linkage disequilibrium mapping by searching for ancestral founder haplotypes around the genes of two single-gene disorders which had been previously mapped: Steinert myotonic dystrophy and pseudo-vitamin D-deficient rickets (Bétard et al., 1995). The results showed that we could have localized the appropriate genes by doing a genome-wide screen with 10 cM or 20 cM spaced markers.

SLSJ population seems to meet the requirement necessary for applying this method, that is, it is shallow in terms of genealogical distances between patients.

5 Application of linkage disequilibrium mapping to Alzheimer disease (AD)

Late-onset Alzheimer's disease has all the characteristics which make it difficult to apply traditional linkage analysis to find its genetic component or components: incomplete penetrance, heterogeneity, phenocopies, etc. It is difficult to propose a model of inheritance for this disease and to define the parameters necessary for linkage analysis. Also the late age-of-onset precludes the collection of families with many living patients over several generations. Linkage to chromosome 19 has been reported, followed by evidence of an association with the E4 allele of the apolipoprotein E gene on this chromosome (Strittmatter et al., 1993; Poirier et al., 1993; Rebeck et al., 1993; Saunders et al., 1993). The apoE4 allele may be a major risk factor for the late-onset form of the disease, but many patients do not carry this allele. Thus, other genes are probably involved as well. To circumvent the problems associated with traditional genetic studies in AD families, we have applied the linkage disequilibrium approach on distantly related AD cases from the population of SLSJ.

METHODS

Selection of a sample of late-onset Alzheimer patients

Selection of a sample of Alzheimer patients was done by means of genealogical analysis. Sixty-three neuropathologically-confirmed late-onset Alzheimer cases, that is, defined as 'senile dementia of the Alzheimer type' (SDAT) were taken from our brain bank. The SDAT diagnosis was established according to a modified Khachaturian scale (Khachaturian et al., 1985). Genealogical data for these SDAT cases was obtained from IREP (Institut Interuniversitaire de Recherches sur les Populations, Chicoutimi, Québec). Ascending pedigrees were reconstructed and analyzed in order to select patients who were related through a limited number of common ancestors at a distance of approximately six generations. First, the minimum number of generations connecting each of the 63 patients with each of the others was determined. Cluster-type analysis provided a dendrogram which summarized genetic distances between groups of patients. Patients too closely or too distantly related were discarded. Genetic contribution of ancestors was also determined in order to identify ancestors who counted among their descendants a high number of SDAT cases (Heyer and Tremblay, 1995). Only descendants from these sources were selected. We obtained a sample of 23 SDAT cases who are all related to each other at an average distance of 5.33 generations. The average age of onset for AD in this sample is 73.7 ± 6.4 years.

distance of approximately 7 cM). A denser map of markers was analyzed in the regions of the presenilin-1 gene (PS-1) on chromosome 14 which is linked to early-onset AD (Sherrington et al., 1995) and of the Apo E gene on chromosome 19. Two types of pedigrees were studied: 1) the case, his or her spouse, and one offspring (n=10); and 2) the case and two offspring (n=13). The spouses' average age when the study began was approximately 84 years and they are all related to each other through their ascending pedigrees at an average distance of 6.25 generations.

10 An association-type analysis was done on the tested markers by estimating the linkage disequilibrium parameter λ (Terwilliger, 1995), a measure of the degree of association or difference in allele frequencies between a group of disease affected persons and a non-disease control group at specific markers. This parameter is 15 mathematically defined in terms of conditional probabilities for allelic frequencies given the absence or presence of a disease chromosome and is estimated using a maximum likelihood approach derived from multinomial probability theory. Dr Lodewijk Sandkuyl (Leiden and Erasmus University, The Netherlands) has modified the LINKAGE ILINK program (Jurg Ott, Columbia University, N.Y., N.Y.) to calculate a maximum likelihood stimat of λ from LINKAGE format pedigree data. This modification performs a two-point analysis (marker and disease locus) for any specified marker. It is capable of deducing non-dis ase-carrying chromosomes to construct a control group. Th 20 analysis was done under th dominance model and th frequency of th disease chromosome was set at 0.01

Haplotypes were reconstructed and the 46 case chromosomes were searched for sharing of multiple successive markers; comparisons were made with the 20 spouse chromosomes and - from the offspring in the type-2 pedigrees - the 13 chromosomes which were 5 transmitted by the non-diseased parent.

DATA ANALYSIS

Data analysis from a genome wide screening of 10 Alzheimer's patients (23), using 700 microsatellites (positioned at an average of 4 to 7 cM), reveals seven (excluding ApoE) different regions in the genome which seem to be implicated in the physiopathology of AD. Genetic markers representing these regions have been sorted with relative P values, and are ordered from greatest importance as follows: 15 D10S212 > D6S273 > D1S228 > D1S232 > Gata89a1 > D2S126 > ApoE > D8S552. Other potential sites of interest have also been detected in the genomic regions containing the Presenilin gene which have previously been shown to be implicated in AD pathology. The P values for these regions, however, were found to be weaker than those observed 20 for the microsatellites listed above.

The microsatellite D10S212 coincides with the region of principal interest as revealed by fin mapping, and is found to be adjacent to an intron of the inositol polyphosphate-5-phosphatase gene (IPP1). This gene encodes a 43-Kda protein involved in the inositol phosphate pathway, its role being that of a downregulator within the 25

Biochemical messengers within most cells effect diverse and complex responses that often depend on the mobilization of Ca^{2+} from intracellular stores within the sarcoplasmic (in muscle) or endoplasmic reticulum (S-ER). Two types of S-ER Ca^{2+} stores have 5 been functionally characterized and identified by immunocyto-chemical localization of receptors (reviewed by Golovina and Blaustein, 1997), and release of Ca^{2+} from one of the stores requires *myo*-inositol 1,4,5-trisphosphate (IP₃).

Two distinct human genes coding for 5-phosphatase 10 (Types I and II) have been cloned, and encode for 43-kDa and 75-kDa proteins respectively. The Type I protein is phosphorylated and activated by protein kinase C, while Type II is not phosphorylated by this kinase. 5-phosphatase enzymes hydrolyze three substrates involved in calcium mobilization: inositol 1,4,5-triphosphate (IP₃), inositol cyclic 1:2,4,5-tetrakisphosphate and inositol 1,3,4,5-tetrakisphosphate (IP₄).

Several studies suggest that alterations in the receptor-mediated phosphoinositide cascade and cytosolic free calcium concentration [Ca^{2+}] are involved in the pathophysiology of aging, and in Alzheimer's disease. Cellular calcium ion signalling is induced by 20 inositol phosphates formed directly or indirectly by the action of phosphatidylinositol-specific phospholipase C on phosphatidylinositol 4,5-bisphosphate in response to extracellular agonists (Berridge and Irvine, 1989; Bansal and Majerus, 1990; Rana and Hokin, 1990). These inositol phosphate signaling molecules are inactivated by inositol polyphosphate-5-phosphatase enzymes (5-phosphatases). Thus, by 25 analogy with the adenylate cyclase/cyclic nucleotide phosphodiesterase

signalling molecules, while the 5-phosphatase acts to degrade them. Changes in the activity of either of these enzymes may alter cellular responses to agonists.

- Three inositol 1,4,5-trisphosphate receptors have so far
- 5 been cloned in humans. They mapped to three different chromosomal regions: the Types 2 and 3 respectively in chromosome 12p11 and 6p21, respectively, and the Type 1 in chromosome 1p. The inositol 1,4,5-trisphosphate receptors (IP₃R) act as IP₃-gated Ca²⁺ release channels in a variety of cell types. The Type 1 receptor (IP₃R1) is the major neuronal
- 10 member of the IP₃R family in the central nervous system. It is predominantly enriched in cerebellar Purkinje cells, but is also concentrated in neurons of the hippocampal CA1 region, caudate-putamen, and cerebral cortex. We have shown recently (unpublished results), that Type 2 and Type 3 receptors are also expressed in specific
- 15 regions of the brain. Matsumoto et al.(1996) have shown that IP₃R1-deficient mice exhibit severe ataxia and tonic or tonic-clonic seizures, and die by the weaning period. Electroencephalograms demonstrate that such mice suffer from epilepsy, indicating that IP₃R1 is essential for proper brain function. Liu et al. (1995), in studies on juvenile myoclonic
- 20 epilepsy (JME) in human families with classical JME, shown that in a region of about 7cM on chromosome 6p21.2-p11 an epilepsy locus exists whose mutated phenotype consists of classic JME with convulsions and/or electroencephalographic (EEG) rapid multispike wave complexes. Again our marker D6S273 is within this interval.
- 25 IP₃R binding sites were studied in autopsied brains from subjects with dementia of the Alzheimer type (DAT) and, in the parietal cortex and hippocampus, there was a 50-70% loss of (³H) IP₃ binding,

whereas no significant changes were observed in frontal, occipital and temporal cortices, caudate or amygdala (L.Trevor Young et al., 1988).

Strikingly, chromosome 1p is not identified as a hot spot by our genetic analysis. This could be explainable by the fact that the 5 founding population did not have a defect on chromosome 1p. Since a type I inositol 1,4,5-triphosphate receptor maps to the chromosome 12p11, IP3R1 should also act as an important target in AD diagnosis and/or treatment. Cloning and analysis of the IP3R1 gene will be carried 10 out to identify mutations or markers associated with AD and CNS disorders in general. The genomic DNA corresponding to the exons and intron/exon junctions of the gene could be amplified using PCR and screened for mutations by the method of single strand conformation polymorphism (SSCP), from which some nucleotide changes have been observed. Experiments employing RT-PCR to analyze this polymorphism 15 on the basis of differential expression levels within a set of patient samples shall also be performed.

All of this data strongly suggests that one or more components of the inositol pathway are considered as excellent candidates for the development of a physiopathological model of 20 Alzheimer disease. In light of the fact that the IP3R1-/- (from human chromosome 1) transgenic mice develop epilepsy, and that studies on human families affected by the JME reveal that the affected loci cosegregate with chromosome 6p21 where the homolog gene (IP₃R3) is located, it appears highly probable that alterations in this pathway could 25 be shared by different forms of genetic neurodisorders. If this proposed scenario is correct, we would expect to find in our population of AD some incidence of epilepsy, and this is indeed the case; the incidence of

epilepsy in our examined population is significantly higher than that normally expected. These observations point tantalizingly towards the hypothesis that various alterations within the inositol biochemical pathway may result in vastly differing phenotypic manifestations, 5 including epilepsy and Alzheimer's disease.

Having now identified the inositol phosphate pathway and more specifically the IP3R1 gene as a key player in CNS disorders and especially in AD, the present invention now permits a biochemical dissection of these diseases. Further, genetic analysis can now be more 10 focussed, and should enable the identification of other genes or products thereof which are part of the pathway or which affect it indirectly. Such analyses should also enable the identification of the critical role of the inositol pathway in other CNS disorders.

The present description refers to a number of documents, the contents 15 of which are incorporated by reference.

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WHAT IS CLAIMED IS:

1. Chromosome 1p gene and gene products related to AD encode a member of the inositol phosphate pathway and use of said chromosome 1p gene and gene products for diagnosis and/or treatment of AD.
2. The Chromosome 1p gene and gene products of claim 1, wherein said Chromosome 1p gene and gene products is IP3R1.